Food allergy: separating the science from the mythology

Per Brandtzaeg

Abstract | Numerous genes are involved in innate and adaptive immunity and these have been modified over millions of years. During this evolution, the mucosal immune system has developed two anti-inflammatory strategies: immune exclusion by the use of secretory antibodies to control epithelial colonization of microorganisms and to inhibit the penetration of potentially harmful agents; and immunosuppression to counteract local and peripheral hypersensitivity against innocuous antigens, such as food proteins. The latter strategy is called oral tolerance when induced via the gut. Homeostatic mechanisms also dampen immune responses to commensal bacteria. The mucosal epithelial barrier and immunoregulatory network are poorly developed in newborns. The perinatal period is, therefore, critical with regard to the induction of food allergy. The development of immune homeostasis depends on windows of opportunity during which innate and adaptive immunity are coordinated by antigen-presenting cells. The function of these cells is not only orchestrated by microbial products but also by dietary constituents, including vitamin A and lipids, such as polyunsaturated omega-3 fatty acids. These factors may in various ways exert beneficial effects on the immunophenotype of the infant. The same is true for breast milk, which provides immune-inducing factors and secretory immunoglobulin A, which reinforces the gut epithelial barrier. It is not easy to dissect the immunoregulatory network and identify variables that lead to food allergy. This Review discusses efforts to this end and outlines the scientific basis for future food allergy prevention.

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Introduction

Allergic reactions to food can affect many organs and, therefore, cause a perplexing variety of symptoms.[1–4] Although nonfood allergies can be diagnosed by well-defined criteria, the diagnosis of food allergy is far more difficult. This is particularly true for the clinical spectrum of food-related allergic manifestations that occur in the digestive tract. Owing to these diagnostic difficulties, it has been difficult to ascertain that food allergy is on the rise in westernized societies to the extent that is documented for other allergic disorders, such as atopic eczema (atopic dermatitis), allergic rhinitis and asthma.[5–7] Prevalence data for adverse reactions to food may be notably affected by an increased public awareness of food allergy. Approximately 25% of the US population believes that they have food allergy and 40–60% of parents think that food causes allergy in their children.[3] However, in westernized societies the prevalence of true food allergy is probably 1–3% in adults and 3–8% in infants.[6,8]

According to a position paper from the European Academy of Allergy and Clinical Immunology (EAACI) published in 1995[9] and subsequently modified,[10] there are two main adverse reactions to food: toxic and non-toxic. The latter comprises pathogenic mechanisms that are both immune-mediated and non-immune-mediated (such as pharmacological, enzymatic and unclear causes, including irritants and psychosomatic responses) (Figure 1). Similar and more elaborate classifications of adverse reactions to food have been proposed by others.[2–4,11]

The immunological or truly allergic reactions to food (Box 1) can be divided into IgE-mediated or non-IgE-mediated. According to the heuristic classification of hypersensitivity that was proposed by Gell and Coombs in the early 1960s,[12] mechanistically, IgE-mediated reactions are considered as type I hypersensitivity and non-IgE-mediated reactions may be tentatively deemed type III hypersensitivity (IgG or IgM immune complex reactions) or type IV hypersensitivity (delayed-type or cell-mediated reactions). Allergic reactions that are a mix of these types of hypersensitivity are likely to exist.[1–4,11] Celiac disease (gluten-sensitive enteropathy) can be considered an allergic disorder because the immunopathology of the lesions present in this condition is driven by type IV and perhaps type III hypersensitivity.[13] However, most clinicians do not consider this condition a food allergy so celiac disease will not be discussed here. The revised EAACI classification,[10] which was adopted by the World Allergy Organization in 2003,[14] introduced the term 'non-allergic food hypersensitivity' for adverse reactions to food in which immunological mechanisms are not identified. This term will not be used here because it is in conflict with the

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classical definition of hypersensitivity that refers to an excessive immune reaction (immunopathology) with undesirable consequences.

The gold standard diagnostic test for food allergies remains allergen exclusion followed by provocation,[15] preferably performed as a so-called double-blind, placebo-controlled food challenge. In children, this challenge is often performed in an open fashion.[4,16] Regardless, even a double-blind, placebo-controlled food challenge cannot always distinguish food allergy from other adverse reactions to food, such as pseudo-allergies (lack of psychological tolerance) or metabolic intolerance. A subgroup of patients with mucosal manifestations of food allergy is characterized by dense eosinophilic mucosal infiltrations and can only be diagnosed by endoscopy and multiple biopsies.[3,11] Children with eosinophilic esophagitis, multiple food allergies or food-protein-induced gastrointestinal syndrome are a particular diagnostic challenge for the pediatric gastroenterologist.[17]

A large proportion (40–50%) of children who are allergic to cow's milk apparently have non-IgE-mediated, delayed-type immune reactions that are perhaps caused by effector T cells or IgG antibodies.[18–20] Classic diagnostic tests for food allergy, such as determining circulating levels of IgE antibodies and the skin prick test (which depends on the detection of IgE antibodies bound to dermal mast cells) are negative in these individuals. However, it is important to be aware that although IgE-induced mast-cell degranulation is central in type I hypersensitivity,[1,21] the terminal effector mechanisms operating in the intestinal lesions of patients with food allergy are difficult to identify with certainty by use of clinical tests or biopsy of the mucosa.[22–25] Mucosal mast cells may, therefore, become sensitized with allergen-specific IgE in mesenteric lymph nodes (MLNs) without such antibodies appearing in blood.[26] Moreover, mast cells are pluripotent cells that not only release potent inflammatory mediators on activation, but might also be involved in the maintenance of the intestinal epithelial barrier.[27]

Key points

- Homeostasis in the gut mucosa is normally preserved by secretory IgA-dependent immune exclusion of antigens and by the suppression of proinflammatory responses to innocuous antigens by mucosally induced tolerance (oral tolerance)
- Food allergy is considered to be the consequence of abrogation of oral tolerance due to inappropriate interactions between genes and the environment
- Any event causing epithelial barrier defects may underlie food allergen sensitization, not only in the gut but also elsewhere in the body, such as the skin and airways
- The successful induction of oral tolerance depends on the dose and timing of enteric exposure to potential allergens, immune-modulating microbial components and dietary factors, such as vitamin A and lipids
- Strict allergen avoidance during pregnancy, lactation, and early childhood to prevent food allergy in families that are genetically at high risk of allergy seems to be based on mythology rather than science
- Exclusive breastfeeding for 4 months and mixed feeding thereafter will probably promote tolerance to food allergens in newborns

This Review will not provide a detailed description of clinical and epidemiological aspects of food allergy, for which the readers are referred to the excellent reviews cited above. Instead, emphasis will be placed on the impact that the rapidly expanding knowledge of mucosal immunology has had on our understanding of the pathogenesis of this complex condition. Science has changed our attitude towards food allergy, which in the past was more clouded by mythology.

The complexity of food allergy

True food allergy is much more frequent in infants and children than in adults and can manifest itself in the skin as atopic eczema or as urticaria; in the skin and mucosa as angioedema; in the respiratory tract as laryngedema or bronchial obstruction, perhaps with wheezing; systemically as anaphylaxis; and in the digestive tract, of which all parts of can be affected from the mouth (oral allergy syndrome) to the anus (proctitis or perianal eczema).[1–4,11,17] Most of the general symptoms of food

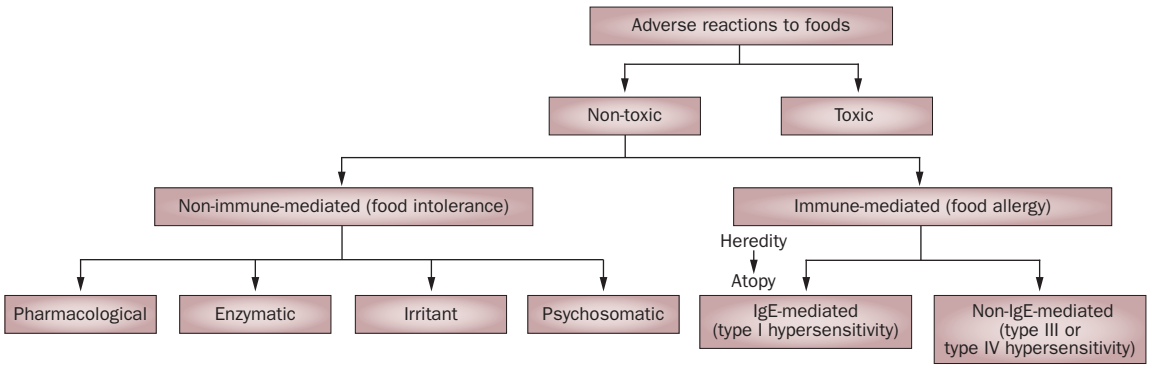

Figure 1 | Classification of adverse reactions to foods according to pathogenic mechanisms.[9] Two main entities of adverse reactions to food exist: toxic and non-toxic reactions. The latter comprises pathogenic mechanisms that are both immune-mediated and non-immune-mediated. Non-immune-mediated mechanisms include pharmacological, enzymatic and unclear causes, such as certain irritants and psychosomatic responses. The IgE-mediated reactions constitute type I hypersensitivity while the non-IgE-mediated reactions are tentatively deemed to be type III hypersensitivity (IgG or IgM immune complex reactions) or type IV hypersensitivity (delayed-type or cell-mediated reactions). Atopy is the hereditary trait of producing excessive levels of IgE antibodies, therefore predisposing to type I hypersensitivity (Box 1).

Box 1 | History and definition of allergy

The term allergy was first introduced by the Viennese pediatrician Clemens von Pirquet in 1906 to describe a "different immunological reaction" which is not protective. The antigens that induced allergies were called allergens. In 1921, Prausnitz & Küstner showed that certain factors in the blood, referred to as reagins, were responsible for the allergic reactions. It took almost 40 years before the reagins were shown to be IgE antibodies. The term atopy was introduced by Coca and Cooke in 1923 to refer to allergy as a "strange familial condition". The recommended definition of atopy is currently: "a personal and/or familial tendency, usually in childhood or adolescence, to become sensitized and produce IgE antibodies in response to ordinary exposures of allergens, usually proteins. As a consequence, these persons can develop typical symptoms of asthma, rhinoconjunctivitis, or eczema".[10,14] However, the terms atopic eczema or atopic dermatitis (the former is used throughout this Review) remain confusing because these terms are often used without detectable atopy;[10,14] eczema is most often not associated with food allergy. Anaphylaxis (meaning "without protection") is also used with different meanings—sometimes it refers to a generalized or systemic allergic reaction and sometimes it is restricted to anaphylactic shock. The recommended definition of anaphylaxis is "a severe, life-threatening, generalized or systemic hypersensitivity reaction".[10,14] In most cases of anaphylaxis a hypersensitivity reaction is involved, but occasionally nonallergic anaphylaxis may be seen.

allergy are unspecific, such as nausea, vomiting, and diarrhea or constipation.[11]

Many allergic reactions to food in adults occur in individuals with pollen allergy because of cross-reactions.[9] A study from Japan published in 2010 showed that almost 5% of individuals who show an IgE response to pollen have an oral allergy syndrome; most of these allergic reactions are associated with eating apple, peach and/or melon.[28] There are also rare case reports of severe hypersensitivity, including anaphylaxis, against mango fruit after the ingestion of fruit salad in individuals with pollen allergy.[29]

To avoid allergic reactions due to excessive penetration of antigens into the intestinal lamina propria, the vulnerable gut mucosa is supported by specialized anti-inflammatory immune defenses, including secretory IgA (SIgA) antibodies and hyporesponsiveness to innocuous agents, particularly dietary antigens and the commensal gut microbiota.[30–32] The induction of these homeostatic mechanisms depends on exogenous stimuli and the neonatal period is particularly critical to this end. Both the intestinal surface barrier with its reinforcement by SIgA and the immunoregulatory network require adaptation. In most cases, this adaptation is remarkably successful in view of the fact that a ton of food, perhaps including 100 kg of proteins,[21] may pass through the gut of an adult human being every year without causing adverse reactions. Classic food allergy reflects a lack of such homeostasis, either due to retarded immunological development with immaturity of the intestinal surface barrier or a persistently imbalanced immunoregulatory network. Both homeostatic deficiencies may be associated with hypersensitivity against innocuous antigens, such as food proteins.

The mainstream theory for the postulated rise in the prevalence of allergies is based on the extended hygiene hypothesis.[33] This thinking underscores the role of environmental changes in immune-mediated disorders and explains why these conditions represent an increasing burden in westernized societies.[33] In essence, the idea is that modern measures introduced in affluent societies have deprived infants of adequate immunological stimuli.[34] Changes in hygienic, dietary and medical practices have altered the pattern of microbial exposure in humans and particularly the composition of the gut microbiota. As discussed later, research into microbial–host interactions aims to reverse immunological hypersensitivity by promoting tolerance.[35–37]

For effective strategies to prevent immune-mediated disorders, including food allergy, it is essential to understand the exogenous variables that influence immunological programming and how they interact with genetic predisposition to disease. Although much of the fundamental research is being carried out in mice, novel ideas are increasingly being supported by experimental human studies and clinical trials.

Mucosal immune regulation

Two strategies of anti-inflammatory defense

Numerous genes regulate innate and adaptive immunity, and human immunogenetics has evolved to identify potential threats under the pressure of a dirty environment. In this evolutionary process the mucosal immune system has generated two anti-inflammatory strategies (Figure 2): immune exclusion—performed by SIgA to control the epithelial colonization of microorganisms and inhibit the penetration of potentially dangerous agents; and hyporesponsiveness—to avoid local and peripheral hypersensitivity against innocuous antigens. Together, these strategies seemingly explain why overt and persistent food allergy are relatively rare. Mucosally induced tolerance against antigens in the gut is referred to as oral tolerance.[38] Similar downregulatory mechanisms also operate against the commensal gut microbiota.[39,40] Differences in these mechanisms may exist between humans and clean laboratory mice due to a more strict shielding of gut bacteria from the systemic immune system in the latter species.[41–44] Oral tolerance is a robust adaptive immune function because substantial amounts of intact food proteins are absorbed by the gut after eating,[26] perhaps amounting to a daily uptake of 130–190 g of food protein.[36]

The intestinal epithelial tightness and the immunoregulatory network remain fragile for a variable period after birth.[45,46] Importantly, animal experiments show that the postnatal development of immunological homeostasis depends on the establishment of a balanced indigenous gut microbiota as well as adequate timing and dosing of the introduction of foreign dietary antigens.[40,43,47] The stimulatory effect exerted by commensal gut bacteria on the regulation and organogenesis of mucosa-associated lymphoid tissue is strikingly revealed in experimental animals that are colonized with a conventional microbiota after being reared in a germ-free state.[40,48,49]

Gut-associated lymphoid tissue

Intestinal immune cells are located in three compartments: organized gut-associated lymphoid tissue (GALT); the mucosal lamina propria; and the mucosal surface

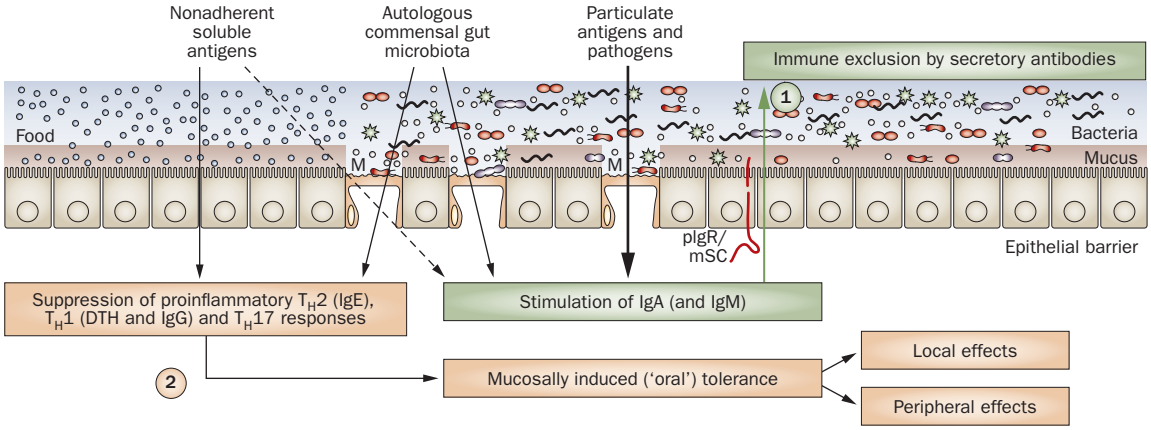

Figure 2 | Anti-inflammatory mucosal adaptive immune defense mechanisms. Two major homeostatic mechanisms preserve the integrity of the intestinal epithelial barrier: (1) productive immunity provides immune exclusion thereby limiting the colonization of pathogens and the penetration of harmful agents. This first line of defense is principally mediated by secretory IgA (and IgM). Secretory antibodies are exported by the pIgR (also called mSC), which is cleaved apically in the epithelium. Mucosal immunity is preferentially stimulated by pathogens and other particulate antigens that are taken up through thin epithelial M cells located above gut-associated lymphoid tissue. (2) Innocuous soluble antigens (for example, food proteins) and the gut microbiota also stimulate secretory immunity (graded arrows indicate degree of stimulation), but in addition induce the suppression of proinflammatory T_H2 responses (the production of IgE antibodies), T_H1-dependent responses (delayed-type hypersensitivity and production of IgG antibodies), and T_H17-dependent granulocytic reactions. The T_H-cell balance is regulated by mucosally induced (oral) tolerance with homeostatic effects both locally and in the periphery. Abbreviations: M cell, membrane cell; mSC, membrane secretory component; pIgR, polymeric Ig receptor; T_H, helper T cell. Permission obtained from Wiley © Brandtzaeg, P. **70,** 505–515 *Scand. J. Immunol.* (2009).

epithelium (Figure 3). GALT comprises Peyer's patches, the appendix and numerous isolated lymphoid follicles.[50,51] The GALT structures represent inductive sites for intestinal immune responses. By contrast, the lamina propria and epithelial compartments principally constitute effector sites for intestinal immune responses, but may nevertheless contribute to the retention, proliferation and differentiation of immune cells.[50,51]

Although GALT is present at birth, it may take a couple of weeks before it becomes clearly activated. This activation is signified by the development of germinal centers.[45] B-cell lymphoid follicles are covered by a specialized epithelium that contains membrane cells, which, together with intraepithelial dendritic cells (DCs), transport antigens from the gut lumen into the lymphoid tissue.[52] GALT structures resemble lymph nodes as they have interfollicular T-cell zones and a variety of antigen-presenting cells (APCs), such as DCs and macrophages, but they are not encapsulated and contain no afferent lymphatic vessels.[53] Antigens, therefore, have to be sampled directly from the intestinal mucosal surface. Induction and regulation of mucosal immunity hence takes place primarily in GALT and draining MLNs, whereas terminal differentiation of B cells to antibody-producing plasma cells occurs in the lamina propria (Figure 3) where secondary T-cell signals are generated when antigens are presented by local DCs.[50] Animal experiments have shown that oral tolerance can be induced in the absence of GALT.[54] This finding indicates that oral tolerance is dependent on antigen transport by DCs to MLNs.[55] However, the induction of oral tolerance may also take place in Peyer's patches, depending on the antigen dose and frequency of feeding.[38,47,56]

Antigens are presented to T cells by APCs after intracellular processing.[31] Activated helper T (T_H) cells release mediators (cytokines), including transforming growth factor-β (TGF-β), which induces the switch of B cells from IgM to IgA expression in GALT follicles.[32,50,51] Memory and effector cells migrate rapidly via efferent lymphatic vessels to MLNs where B cells are further differentiated to plasmablasts, which then reach the peripheral blood via the thoracic duct and finally become seeded into secretory effector sites (Figure 3). This homing mechanism particularly targets the intestinal lamina propria but to some extent also distant mucosae and, notably, the lactating mammary glands.[57] The local extravasation of plasmablasts is facilitated by compartmentalized homing receptors that interact with ligands (addressins) on the microvascular endothelium and additional fine-tuned navigation is conducted by chemokines.[32,50,51] The extent of B-cell retention and terminal differentiation to plasma cells depends on the intensity of local signals provided by chemokines and DC-processed antigens via activated $CD4^+$ T_H cells and their cytokines (Figure 3).

Postnatal immune development

Very few IgA^+ plasmablasts circulate in the blood of newborns (<8 per million mononuclear cells) although this number increases remarkably (to ~600 per million mononuclear cells) after 1 month, which reflects the progressive microbial stimulation of GALT.[58] An initial early increase in levels of circulating plasmablasts (mainly the IgM^+ phenotype) occurs in preterm infants, especially infants with intrauterine infections.[59] Mucosal immune cells are, therefore, competent at least during the final trimester, but APCs need to be activated by microbial factors

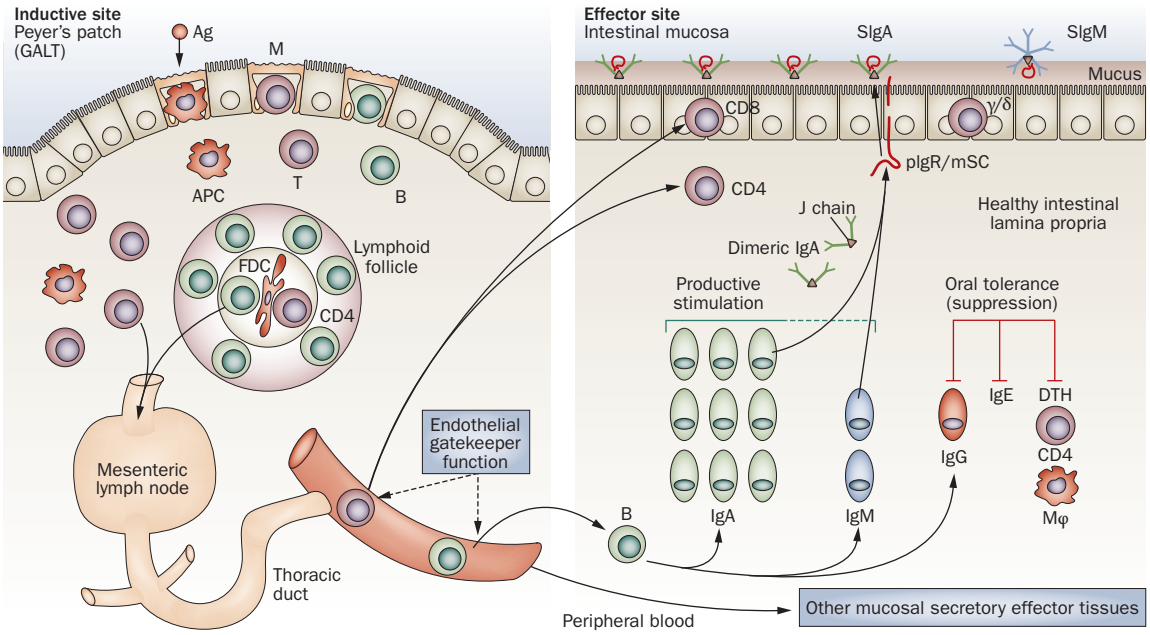

Figure 3 | The intestinal immune system. Inductive sites for mucosal T cells and B cells are constituted by GALT (including Peyer's patches) which contains M cells that transport antigens to APCs (dendritic cells, Mφ and FDCs). Activated naive T cells and B cells become memory and effector cells, which migrate to mesenteric lymph nodes and then via the thoracic duct to peripheral blood for extravasation at mucosal effector sites. Microvascular endothelial cells express adhesion molecules and chemokines for gut homing and exert a 'gatekeeper function' for mucosal immune cells. Healthy intestinal lamina propria (effector site) is illustrated with its relative proportions of IgA^+, IgM^+ and IgG^+ plasma cells, $CD4^+$ T cells and Mφs. Intraepithelial lymphocytes ($CD8^+$ and γ/δ^+ T cells) are depicted. SIgA and SIgM are exported to the mucus via pIgR/mSC.[32,50] Oral tolerance mechanisms control the pathogenic effects of proinflammatory antibodies (IgG and IgE) and cell-mediated ($CD4^+$ T cell and Mφ) DTH. Abbreviations: Ag, antigen; APC, antigen-presenting cell; B, B cell; DTH, delayed-type hypersensitivity; FDC, follicular dendritic cell; GALT, gut-associated lymphoid tissue; J chain, joining chain; M, membrane cell; Mφ, macrophage; mSC, membrane secretory compenent; pIgR, polymeric Ig receptor; SIgA, secretory IgA; SIgM, secretory IgM; T, T cell. Permission obtained from Wiley © Brandtzaeg, P. **70**, 505–515 *Scand. J. Immunol.* (2009).

that enable them to provide appropriate co-stimulatory signals to naive T cells.[60] The commensal gut microbiota is important in this context as shown by the fact that the number of intestinal IgA^+ plasma cells is normalized 4 weeks after exposure of germ-free mice to a conventional gut microbiota.[61] *Bacteroides* and *Escherichia coli* strains seem to be particularly immunostimulatory, but lactic-acid-producing bacteria also contribute.[62,63]

The slow postnatal activation of GALT parallels the functionally decreased systemic immunocompetence of newborns.[45–47,60] Peripheral $CD4^+$ T_H cells in infants show reduced capacity for cytokine production and provide poor B-cell help compared with in adults.[60] One reason for this reduced immunocompetency is that there are relatively few circulating immune cells in infancy.[64] Moreover, deficiencies in the numbers of APCs and their functional competence prohibit the induction of efficient memory responses.[65] This deficiency normally affects the T_H1 responses, which *in utero* are mainly associated with abortion, whereas the secretion of T_H2 cytokines generally reflects a normal pregnancy.[66]

Epithelial barrier function

Reinforcement by secretory antibodies

In adults, the intestinal mucosa contains at least 80% of the body's plasma cells, most of which produce dimeric IgA, including a polypeptide called joining (J) chain.[32,50] This product becomes SIgA after export by the epithelial polymeric Ig receptor (pIgR), also known as membrane secretory component (mSC).[32,67] Smaller amounts of J-chain-containing pentameric IgM are also exported by the pIgR, but SIgM is not as stable as SIgA because the cleaved receptor (called bound SC) is covalently stabilized only in the latter. Immune exclusion at the intestinal mucosal surface barrier is, therefore, normally performed by SIgA (Figure 3), which cooperates with innate defenses, such as the mucus layer, defensins and peristalsis.[30,44,68] SIgA, which is mucophilic because of its bound SC, may interact with mucin to keep bacteria and other antigens away from the intestinal epithelium by forming a biofilm, as observed in the mouse gut.[69]

In newborns and individuals with selective IgA deficiency, SIgM antibodies probably have a more important role in mucosal defense.[45,50] IgA is generally undetectable in the mucosa before 10 days of age and IgM^+ plasma cells often remain predominant for up to 1 month. Thereafter, a rapid expansion of IgA^+ plasma cells takes place, and some increase may be seen even after 1 year of age. Accordingly, only traces of SIgA and SIgM usually occur in intestinal fluid during the initial postnatal period, whereas some IgG is often present. The presence of IgG reflects paracellular leakage from the intestinal lamina

propria of the newborn, which after 34 weeks of gestation contains readily detectable maternal IgG.[45] A much faster establishment of SIgA immunity may be seen in newborns in developing countries because of the presence of a heavy microbial load.[70] Reports suggest that probiotic treatment enhances the production of IgA, but this finding has not been confirmed by tests measuring the presence of IgA in saliva.[71] Nevertheless, a study has shown that the combination of a prebiotic and probiotic (that is, a synbiotic) treatment given perinatally for 6 months to infants increased levels of fecal IgA and reduced risk of allergy, including food allergy, before 2 years of age.[72]

Uptake of SIgA from mother's milk via the neonatal gut mucosa is negligible and of no importance for systemic immunity,[73,74] except perhaps in preterm infants.[75] Although the physical maturation and sealing of the intestinal epithelium, or so-called 'gut closure', normally occurs in humans before birth, the intestinal surface barrier may be inadequate up to 2 years of age. The mechanisms involved in enhancing epithelial barrier function remain poorly defined,[76] but the development of adequate secretory immunity is probably an important aspect.

Importantly in this context, pIgR-deficient mice that lack SIgA and SIgM exhibit aberrant mucosal leakiness in the gut[77] and increased uptake of food antigens and commensal intestinal bacteria.[78] These animals show a generalized hyper-reactive immune state with overactivation of the cellular NFκB transcription pathway that results in a 50% chance of IgG-dependent anaphylactic death after systemic antigen sensitization to ovalbumin and low-dose intradermal challenge with the same antigen.[79] However, these animals also show an enhanced capacity for the induction of oral tolerance. After feeding ovalbumin to these mice, IgG1 antibody production and T-cell-mediated hypersensitivity were controlled, resulting in complete protection against anaphylaxis (Figure 4). This observation might imply that although an inadequate intestinal surface barrier in newborns represents a risk of allergy, it promotes mucosally induced tolerance against antigens when continuously present in the gut. The balance between the mucosal barrier function and oral tolerance may, therefore, be critical.

Studies have suggested that immune hyper-reactivity may even result from intrauterine influences in newborns who subsequently develop allergy; such an adverse development is probably due to genetically or epigenetically determined poor regulatory T (T_{REG})-cell function and immunological immaturity.[80,81] This concept is supported by studies of T cells from the cord blood of newborns with a potential genetic predisposition to allergy.[82] Conversely, although the incidence of food allergy (apparently non-IgE-mediated) is found to be raised in children with selective IgA deficiency, it is not strikingly increased.[83] This finding may be explained by enhanced induction of T_{REG} cells in addition to compensatory SIgM, which partially replaces the lack of SIgA in the gut.[84]

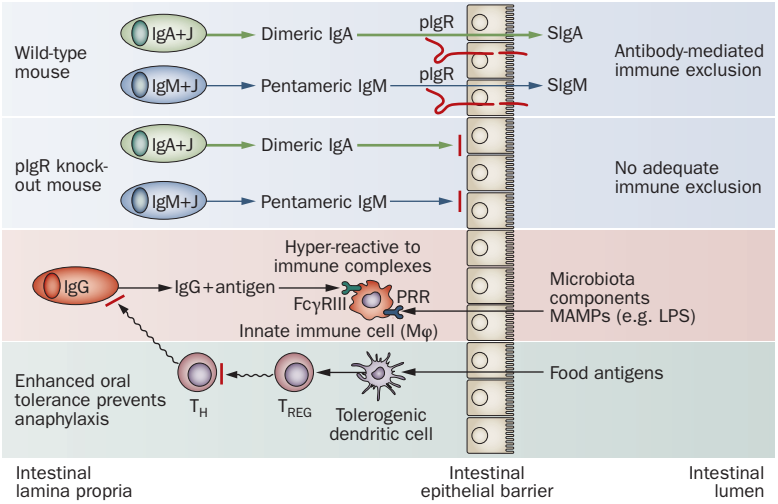

Figure 4 | Depiction of an experimental mouse model of interactions between oral tolerance and immune hypersensitivity. Lack of SIgA and SIgM in pIgR knockout mice leads to a deficient intestinal epithelial barrier and inadequate immune exclusion of products from the gut microbiota. Conserved MAMPs (for example, LPS) bind to PRRs on innate immune cells, such as Mφs, which become hyper-reactive. These cells are, therefore, sensitive to IgG-containing immune complexes that interact with FcγRIII and render the mice predisposed to anaphylaxis. The deficient intestinal epithelial barrier also allows the increased uptake of food antigens (for example, fed ovalbumin). This process enhances the induction of oral tolerance thereby providing a net anti-inflammatory effect against undue body access of the same antigen by any route (for example, dermal). Production of IgG antibodies against this sensitizing antigen is downregulated and the animal is protected against anaphylaxis after antigen challenge. Abbreviations: FcγRIII, Fc receptors for IgG; J, joining chain; LPS, lipopolysaccharide; Mφ, macrophage; MAMP, microbe-associated molecular pattern; pIgR, polymeric Ig receptor; PRR, pattern recognition receptor; SIgA, secretory IgA; SIgM, secretory IgM; T, T cell; T_H, helper T cell; T_{REG}, regulatory T cell. Permission obtained from Wiley © Karlsson, M. R. *et al. Allergy* **65**, 561–570 (2009).

Role of commensal gut bacteria

Experiments in mice have demonstrated the crucial role of commensal gut bacteria in establishing and regulating the intestinal surface barrier, including the upregulation of pIgR expression.[85,86] The beneficial effects of these bacteria seem to be largely mediated by pattern recognition receptors (PRRs), particularly Toll-like receptors (TLRs), which are expressed by the gut epithelium.[87] The conserved microbial ligands are preferably called microbe-associated molecular patterns (MAMPs),[43] although they were previously referred to as pathogen-associated molecular patterns (PAMPs).

Polarized epithelial cells have the ability to dampen the proinflammatory effect of PRR-mediated signals coming from the luminal side.[86,88] However, after bacterial invasion, PRR signaling from the basolateral side results in NFκB activation and the release of epithelial defensins to combat the infection.[87,89] Accumulating evidence suggests that barrier-related homeostasis depends on crosstalk between the epithelium (via cytokines and other factors) and lamina propria cells, including DCs, macrophages and T cells.[90–92] When immune regulation is operating correctly, the small amounts of antigen that reach the lamina propria are handled in a homeostatic manner by DCs and macrophages with balanced cytokine secretion and the induction of T_{REG} cells (Figure 5). However, if the

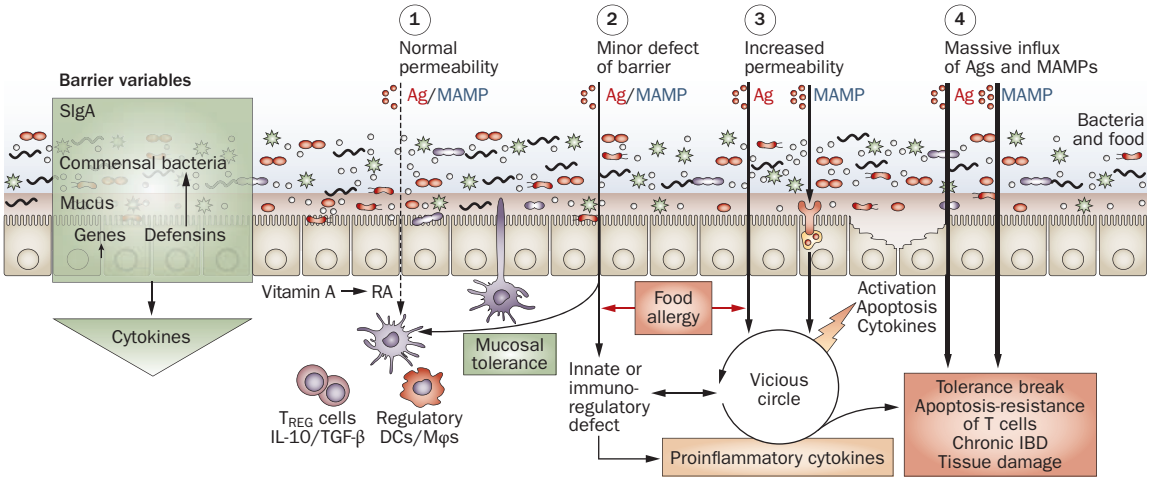

Figure 5 | Maintenance of mucosal homeostasis in the gut and the abrogation of oral tolerance. (1) Normal epithelial permeability allows some uptake of food Ags and MAMPs. Homeostasis is maintained by epithelial-cytokine-conditioned DCs and Mφs that convert dietary vitamin A to RA, which aids the induction of T_{REG} cells with the suppressive cytokines IL-10 and TGF-β. (2) A minor barrier defect (variables one the left) results in increased uptake of Ag and MAMPs but with maintained homeostasis. (3) If an innate or regulatory defect exists, a vicious circle develops that acts reciprocally on the homeostatic network. The epithelium is activated and Ag and MAMP uptake is enhanced both by increased permeability and apical receptor expression. The degree of Ag and MAMP uptake is reflected by the thickness of the vertical arrows in the different stages of intestinal permeability. Food allergy probably develops between stages 2 and 3 and can potentially be reversed. (4) Adverse progression results in epithelial overactivation, apoptosis, and increased secretion of proinflammatory cytokines that lead to apoptosis-resistant effector T cells, chronic IBD and tissue damage. Abbreviations: Ag, antigen; DC, dendritic cell; Mφ, macrophage, MAMP, microbe-associated molecular pattern; RA, rentinoic acid; T_{REG}, regulatory T cell; SIgA, secretory IgA.

antigenic influx is excessive or immunoregulation is defective, immune reactions may be driven into hypersensitivity and enter a vicious circle of proinflammatory cytokine secretion and epithelial cell apoptosis.[91,93,94] A point of no return may be reached, as occurs in patients with IBD.

In fetal life, murine gut epithelial cells are sensitive to microbial factors, such as lipopolysaccharide, because they express a PRR for this MAMP, namely TLR4.[95] Exposure to lipopolysaccharide in the vaginal tract during birth temporarily activates the neonatal gut epithelium so that it becomes tolerant to MAMPs because of suppressed TLR signaling. By remarkable contrast, such epithelial homeostasis does not occur in mice delivered by cesarean section.[95] These experimental findings are in line with the observation that children delivered by cesarean section seem particularly prone to develop food allergy if they have a genetic predisposition for atopy.[96]

Breastfeeding and mucosal immunity

Counteracting immune dysfunction

SIgA seems to be one variable that contributes substantially to an individual's threshold for exhibiting allergy to food (Figure 5). Evidence suggests that allergic reactions are related to a retarded development of IgA-producing cells or insufficient SIgA-dependent function of the intestinal surface barrier.[56] Indeed, minor dysregulations of both innate and adaptive immunity (especially low levels of IgA) have been observed in children with multiple food allergies.[97] In line with this observation, exclusive breastfeeding up to the age of at least 4 months seems to have an allergy-preventive effect both in families with or without a predisposition to allergy.[98–100] Moreover, to avoid IgE sensitization and food allergy, it seems to be favorable to introduce nutritionally adequate, safe and appropriate complementary foods at around 4 months of age with maintained breastfeeding for at least 2 more months.[101,102]

In addition to the remarkable reinforcement of mucosal defense provided by maternal SIgA (and SIgM) antibodies as a natural immunological substitution therapy or passive immunization, it is important to emphasize the positive nutritional effect of breastfeeding on immune development.[56] Breast milk contains a number of immune cells, cytokines and growth factors that may exert many beneficial biological effects in the breastfed infant's gut.[56,57,103]

Several studies of the effect of breastfeeding on the development of secretory immunity have been performed by use of salivary IgA measurement, but variable results have been reported. Sample contamination with milk SIgA, shielding of the suckling's mucosal immune system by maternal SIgA antibodies and altered growth and composition of the infant's gut microbiota have been discussed as possible uncontrollable variables that might lead to discrepant results.[45]

Altogether, many prospective studies have reported that the initial postnatal increase of salivary IgA (and IgM) in newborns is more prominent in formula-fed than in solely breastfed infants.[45] Nevertheless, the balance of evidence suggests that breastfeeding over time (up to 8 months) promotes the development of secretory immunity at several mucosal effector sites.[56,57,103]

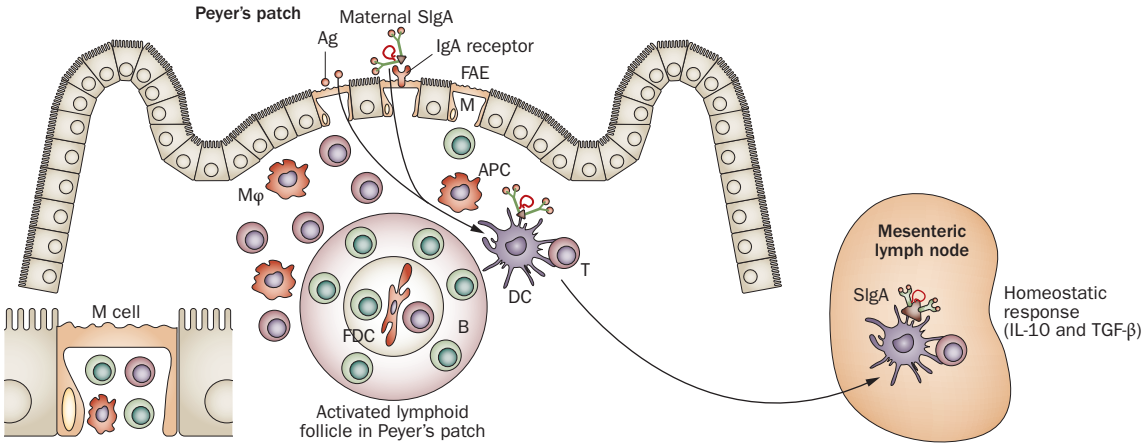

Figure 6 | Putative integration of maternal SIgA into the immune system of a breastfed infant via M-cell-mediated Ag uptake. Maternal SIgA antibodies in breast milk may guide induction of the infant's intestinal immune system because M cells in Peyer's patches express an as yet uncharacterized receptor for IgA. This receptor facilitates uptake of Ags that have formed immune complexes with cognate maternal SIgA antibodies in the gut lumen of the infant. The complexes may be targeted to infant dendritic cells (DCs) that carry them to mesenteric lymph nodes where a homeostatic immune response dominated by the secretion of TGF-β and IL-10 is induced. Based on experimental data.[104] An M cell with its cellular content is schematically shown in the left panel. Abbreviations: APC, antigen-presenting cell; B, B cell; FAE, follicle-associated epithelium; FDC, follicular dendritic cell; Mφ, macrophage; M, membrane cell; SIgA, secretory IgA; T, T cell.

One possibility is that maternal SIgA antibodies may guide the uptake of corresponding dietary and microbial antigens via receptors for IgA on membrane cells. This putative mechanism may provide relevant stimulation of the breastfed infant's immune system (Figure 6). Experiments in mice suggest that antigens may be targeted to DCs, which migrate to MLNs where they induce a homeostatic immune response.[104]

Breastfeeding and oral tolerance induction

The induction of oral tolerance clearly involves more than one mechanism and experimental data suggest that extensive biological complexity exists (Figure 7). Important variables with regard to oral tolerance induction include genetics, age, dose and timing of postnatal oral antigen administration, antigenic structure and composition, gut epithelial barrier integrity, and the degree of concurrent local immune activation (reflected by local cytokine profiles and expression of co-stimulatory molecules on APCs).[38,47,56] In addition, the suppressive effects of various subsets of T_{REG} cells induced by homeostatically conditioned APCs, particularly mucosal DCs and macrophages, are being increasingly investigated for their role in oral tolerance induction.

By dampening early mucosal immune activation (for example, by reducing the APC expression of the co-stimulatory B7 molecules, designated CD80 and CD86), the shielding effect of maternal SIgA on the breastfed infant's GALT may contribute to systemic-type hyporesponsiveness against commensal gut bacteria and dietary antigens.[56] SIgA antibodies to food constituents are present in breast milk, and cow's milk allergy is more likely to develop in infants whose mothers have relatively low antibody levels against bovine proteins.[105,106] The presence in breast milk of the cytokines TGF-β and IL-10 might further contribute to oral tolerance not only because of their immunosuppressive effect, but also by promoting mucosal IgA induction.[32,56,57,107] Furthermore, a direct enhancing effect on the intestinal epithelial barrier has been reported for TGF-β.[108] Finally, as discussed above,[98–102] epidemiological data support the view that breastfeeding protects newborns against allergic disorders, such as food allergy, atopic eczema and asthma.

Together, these observations support the notion that the intestinal surface barrier of infants is reinforced by breastfeeding and this seems to be particularly important in families with a genetic predisposition to allergy.[109] A tentative conclusion might be that breast milk together with complementary foods after 4 months (see above), rather than abrupt weaning, seems to promote tolerance to food proteins.[101,102]

Small amounts of foreign proteins transferred into breast milk might further promote tolerance in breastfed infants. This was first suggested experimentally in a mouse model of ovalbumin-induced asthma where the antigen appeared in the mother's milk and protected against asthma in offspring by the generation of T_{REG} cells.[110] A similar study of ovalbumin-induced airway disease in mice showed that the induction of tolerance in the offspring required mothers to have B cells;[111] therefore, it is possible that antibody-dependent epithelial uptake of the transferred antigen takes place in the neonate's gut, as discussed above for membrane cells (Figure 6). Importantly, tolerance induction was also shown in the offspring of mice that experienced low-dose peanut exposure in the gut during pregnancy and lactation; the pups were protected against IgG1-dependent anaphylaxis and showed reduced levels of peanut-specific IgE upon intragastric antigen challenge.[112] These results go against previous myths that early allergen avoidance is crucial for allergy prevention, as discussed later.

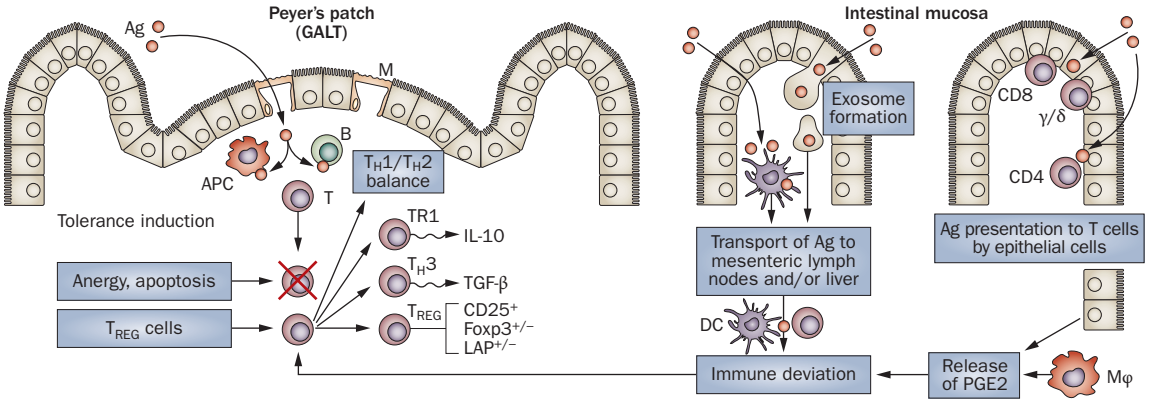

Figure 7 | Putative mechanisms of oral tolerance induction. Hyporesponsiveness to innocuous Ags may be explained by T-cell anergy, clonal deletion by apoptosis and active (contact-dependent) or cytokine-mediated (immune deviation) suppression exerted by subsets of T_{REG} cells. T_{REG}-cell induction occurs locally or at distant sites, such as the lymph nodes or the liver[256,257] after the dissemination of soluble absorbed Ags or the transport of Ags by APCs or epithelial exosomes. Mucosal and peripheral hyperactivation of effector T cells is avoided by the induced T_{REG} cells. $CD25^+$ T_{REG} cells are either positive or negative for Foxp3, and certain subsets (T_R1, T_H3, or LAP^+) produce the suppressive cytokines IL-10 and TGF-β. T_{REG} cells are important for the development of a balanced T_H1/T_H2 cytokine profile. Immune suppression in the gut may also be driven by unconventional Ag presentation by epithelial cells to intraepithelial or subepithelial T cells of various phenotypes ($CD8^+$, γ/δ^+, $CD4^+$) and the immune deviation effect of PGE2 released from APCs or the epithelium. Abbreviations: Ag, antigen; APC, antigen-presenting cell; B, B cell; DC, dendritic cell; M; membrane cell; Mφ, macrophage; GALT, gut-associated lymphoid tissue; PGE2, prostaglandin E2; T, T cell; T_H, helper T cell; T_{REG}, regulatory T cell.

Mucosal homeostasis and allergy

Human tolerance to dietary antigens

Epidemiological data suggest that nearly 4% of children in the US have food allergy, and peanut allergy has doubled in children below 5 years of age in the second half of the past decade.[2] In the US, food allergy accounts for about 30,000 anaphylactic reactions, 2,000 hospitalizations and perhaps up to 200 deaths each year.[3] These data suggest that an anaphylactic epidemic has emerged.[113] Such lack of oral tolerance may be the starting point for subsequent allergic airway diseases, particularly in atopic individuals.[10]

Oral tolerance is thought to be largely explained by different T-cell events, such as anergy, clonal deletion and the induction of T_{REG} cells by conditioned APCs (Figure 7), although other regulatory principles may be involved.[47,56,114–116] However, for ethical reasons, the existence of oral tolerance in humans is supported mainly by circumstantial evidence. The gut mucosa of healthy individuals contains virtually no hyperactivated T cells, hardly any proinflammatory IgG production, and serum levels of IgG antibodies to food antigens are low.[56] The systemic IgG response to dietary antigens tends to decrease with increasing age.[117,118] Moreover, a hyporesponsive state to bovine serum albumin has been demonstrated by intradermal testing in adults, suggesting an effect of mucosally induced tolerance to this cow's milk protein.[119] Interestingly, nasal application or feeding of a novel antigen (keyhole limpet hemocyanin) to healthy individuals has been shown to induce peripheral downregulation of T-cell immunity and, less consistently, suppress systemic antibody responses to subsequent parenteral immunization against the same novel antigen.[120,121] By contrast, oral tolerance is not induced in patients with IBD in whom the intestinal surface barrier is severely deteriorated[122] and data suggest that a genetic defect in oral tolerance induction exists in such individuals.[123]

Microbial recognition and induction of T_{REG} cells

Resident APCs in healthy human gut mucosa are quite inert in terms of their ability to stimulate a productive immune response[124] and they do not express detectable surface levels of TLR2 or TLR4.[125] Furthermore, only negligible levels of the lipopolysaccharide receptor CD14 are normally present on these cells and their proinflammatory cytokine response is usually low after lipopolysaccharide stimulation.[125,126]

These observations support the notion that both macrophages and DCs have a central role in oral tolerance.[90] Indeed, most human mucosal APCs come from a common myeloid progenitor and often show an intermediate phenotype, so it is often not possible to distinguish between macrophages and DCs in the human gut.[127] Heterogeneity of murine lamina propria APCs has also been reported.[128,129] In a quiescent steady state, mucosal $CD103^+$ $CCR7^+$ DCs (and possibly macrophages) carry penetrating dietary and innocuous microbial antigens away from the intestinal mucosa to the MLNs;[55,130] here the same cells, in a maturation process, become further conditioned for tolerance induction and drive the expansion of T_{REG} cells.[47,56,131] Notably, the phagocytic and bacteriocidal activity of the intestinal macrophages is maintained,[132] which is important for the silent clearance of commensal gut bacteria that normally penetrate into the gut mucosa in small numbers.[42] In combination, these homeostatic mechanisms protect against hyperactivation of mucosal effector T cells and accompanying inflammation. Homeostatic control is also exerted when T_{REG} cells, directed by their surface

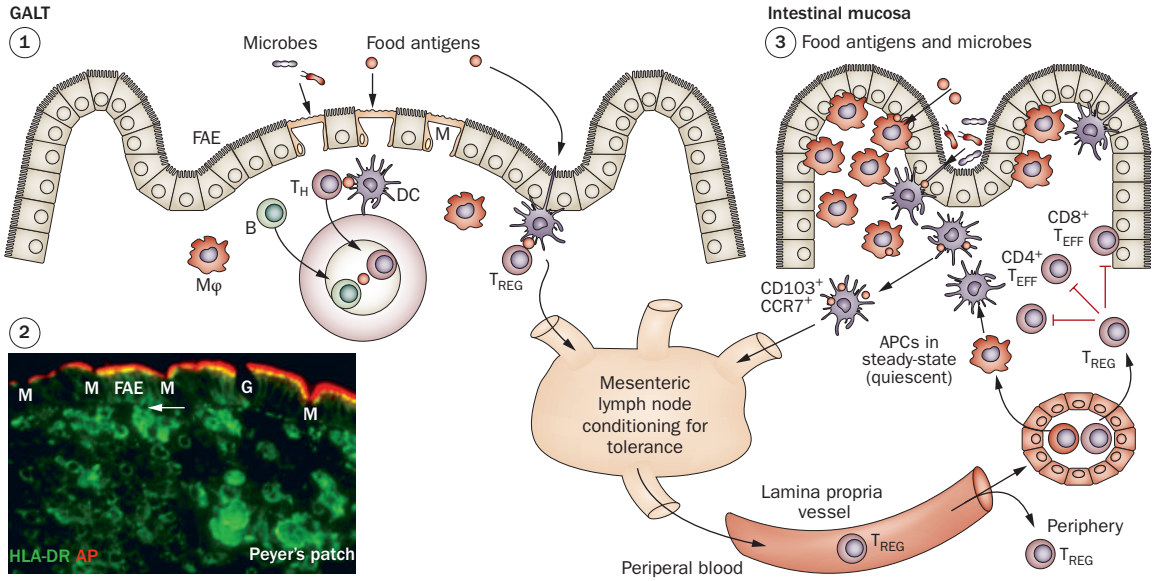

Figure 8 | Conditioning of APCs for tolerance induction. (1) Distribution of APCs below the FAE in a Peyer's patch (GALT). (2) The distribution of APCs below the FAE is shown by paired immunofluorescence for HLA-DR where M and occasional G cells are demarcated by lack of AP. (3) Some APCs extend their dendrites between epithelial cells to sample luminal antigens. Such dendrites can also be seen in the FAE of GALT. Subepithelial APCs, mainly $CD103^+CCR7^+$ DCs with captured antigen, migrate via draining lymph to mesenteric lymph nodes where they either mature to become active APCs that stimulate T_{EFF} cells for productive immunity (arrow) or become conditioned for tolerance via the generation and/or expansion of T_{REG} cells. These inducible T_{REG} cells migrate via efferent lymph to peripheral blood and then to the mucosa or the periphery where they exert anti-inflammatory control of $CD4^+$ and $CD8^+$ T_{EFF} cells. Abbreviations: AP, alkaline phosphatase; APC, antigen-presenting cell; B, B cell; DC, dendritic cell; FAE, follicle-associated epithelium; G, goblet cell; GALT, gut-associated lymphoid tissue; M, membrane cell; Mφ, macrophage; T_{EFF}, effector T cell; T_H, helper T cell; T_{REG}, regulatory T cell.

integrin α4β7 and other gut-homing molecules, migrate from MLNs to the intestinal lamina propria (Figure 8). The gut homing mechanisms seem particularly favorable for T_{REG} cells in infancy.[133]

A dietary effect on the induction of T_{REG} cells is exerted by the conversion of vitamin A to retinoic acid. This conversion depends on retinal dihydrogenase (RALDH), which is expressed by both intestinal DCs, macrophages and epithelial cells,[138,134] as well as by MLN stromal cells.[135] Retinoic acid is not only important for the induction of gut-homing molecules but, together with IL-2, TGF-β and IL-10, can drive the development of T_{REG} cells,[136–139] which may differentiate from rapidly proliferating memory T cells.[140]

Tolerance induction in MLNs (Figure 8) seems to be favored by appropriate stimulation of migrating APCs by certain MAMPs (Figure 9). MAMPs include conserved cell wall products of commensal gut bacteria and components of parasites, such as helminths.[33,43,141–143] For example, several studies suggest that lipopolysaccharide has a central role in the early programming of the immune system[144,145] and research is ongoing to find out if allergy to food and aeroallergens is associated with mutations (polymorphisms) in PRRs that recognize lipopolysaccharide and other MAMPs, such as CD14, TLR2, TLR4 and NOD.[142,143] The extended hygiene hypothesis implies that suboptimal PRR stimulation with delayed maturation of the mucosal immune system and insufficient induction of T_{REG} cells contributes significantly to the increasing incidence of not only allergy—commonly reflecting overactivation of T_H2 cells—but also other immune-mediated inflammatory disorders—reflecting overactivation of T_H1 or T_H17 cells (Figure 9). T_H1 and T_H17 responses are normally important for the defense against infections[33,43] and, from an evolutionary perspective, T_H2 responses have had a crucial role in host protection against parasites.[148,149]

This basis for the hygiene hypothesis has been tested in several clinical investigations. These studies have evaluated the beneficial effect of probiotic bacterial preparations derived from commensal gut microbiota[33] and eggs of the porcine helminth (whipworm) *Trichuris suis* on immune homeostasis.[143] In this context, viable strains of lactobacilli and bifidobacteria have been reported to enhance IgA in humans and animals, but these responses have not translated convincingly into clinical effects.[150] The selection of safe and effective probiotic strains or synbiotic combinations with prebiotics or breast milk remains difficult.[151]

Attempts are also being made to explore the effect that DNA from probiotic lactic-acid-producing bacteria might exert on the integrity of the intestinal epithelium through interaction with TLR9.[152] A specific TLR9 ligand, namely a synthetic noncoding unmethylated cytosine-guanine (CpG) 6 bp DNA sequence, has been tested and shown promising clinical results in individuals suffering from ragweed-induced allergic rhinitis.[153] This DNA sequence is highly enriched in bacteria, but only future studies will show if it has a therapeutic potential in patients with food allergy.

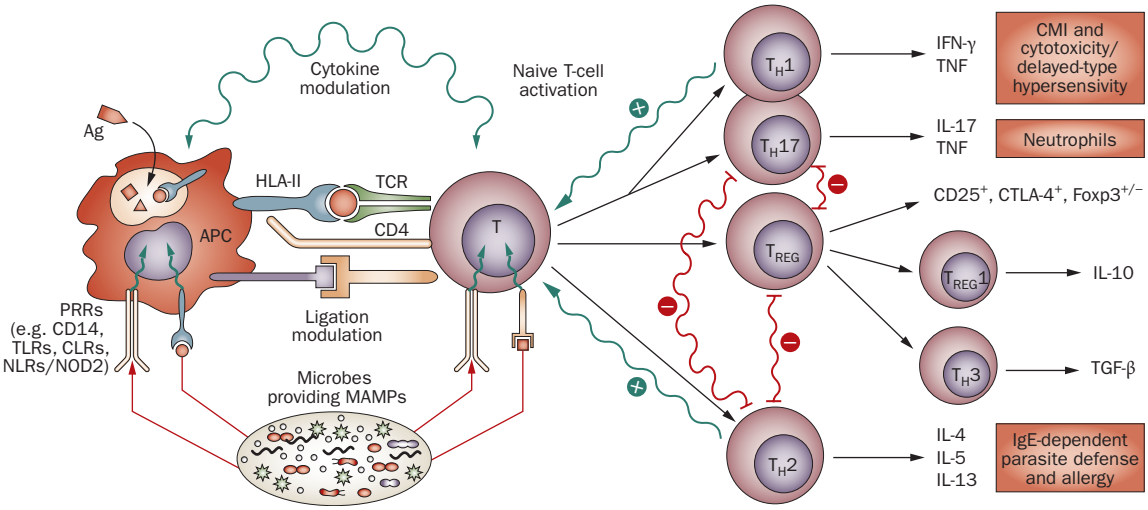

Figure 9 | T-cell activation and differentiation. This process is modulated by co-stimulatory signals (cytokines and ligands) between APCs and naive (or memory) T cells. Naive CD4+ T-cell activation occurs when an APC takes up and degrades an Ag for display to the TCR in the polymorphic HLA-II groove of the T cell. Activated CD4+ T cells differentiate into T_H1, T_H17 or T_H2 effector cells. Cytokine secretion by these cells depends on signals from MAMPs that are sensed by PRRs on the surface of APCs and T cells. PRR signaling to the nucleus of these cells stimulates their activation and maturation and dictates the differential expression of co-stimulatory molecules that direct polarized T_H cytokine profiles, which are further promoted by positive (green waved arrows) and inhibitory (red waved lines) feedback. The cytokines produced induce defenses or immunopathology as indicated (red panels). APCs can also induce various subsets of T_{REG} cells that by means of regulatory molecules and suppressive cytokines can inhibit T_H cell responses and inflammation. Abbreviations: Ag, antigen; APC, antigen-presenting cell; CMI, cell-mediated immunity; HLA-II, HLA class II molecule; IL, interleukin; MAMP, microbe-associated molecular pattern; PRR, pattern recognition receptor; TCR, T-cell receptor; T_H, helper T cell; T_{REG}, regulatory T cell.

It remains unknown whether probiotics in the gut might work to improve mucosal homeostasis through SIgA-dependent reinforcement of the intestinal surface barrier, the expansion of T_{REG} cells, or the involvement of both of these anti-inflammatory mechanisms, perhaps combined with direct PRR-mediated strengthening of epithelial integrity (Figure 5). Notably, the most promising results in studies of food allergy have been reported in studies that have used atopic eczema as an outcome measure.[150] This skin disease is often seen in IgE-mediated food allergy (20–40% of patients) (Box 1) and is particularly associated with loss-of-function mutations in the fillagrin gene, which is involved in the epidermal barrier function.[154] Similar mutations seem to predispose individuals to the combination of atopic eczema and asthma.[155] These findings apparently indicate that a leaky surface epithelium anywhere in the body may be a predisposing condition for allergen penetration and that food allergy could be a consequence, rather than a cause, of atopic eczema. The use of atopic eczema as a marker of food allergy may, therefore, belong to mythology.

Innate decision-making dictates homeostasis

Microorganisms have inhabited Earth for at least 2.5 billion years and the power of the immune system is a result of coevolution in which commensal gut bacteria have shaped host defense mechanisms in a state of mutualism.[33,43] Mucosal homeostasis in the gut is indeed remarkable because of the large surface area that requires defense, some 300 m^2 in adults, and the large number (more than 1,000) of indigenous and transient bacterial species.[156] The gut microbiota is estimated to be composed of ~10^{14} bacteria (approximately 10 times the number of body cells) and weighs 1–2 kg.[43] The gut microbiome is perhaps 150 times larger than the human genome. Notably, although the gut provides the largest area in the body for exposure to colonizing bacteria, it also provides major exposure to foreign proteins that could be potential allergens and micronutrients that have immunomodulatory properties (Figure 10).

The original hygiene hypothesis postulated that the increasing incidence of allergy in westernized societies was explained by reduced or aberrant microbial exposure early in infancy. This reduced immunostimulation resulted in too little T_H1 cell activity and, therefore, an insufficient level of IFN-γ to cross-regulate IgE-inducing T_H2 cell responses.[34] The composition of the gut microbiota and exposure to food-borne and orofecal pathogens probably have important homeostatic influences,[157,158] both by enhancing the SIgA-mediated intestinal surface barrier and by promoting oral tolerance through a shift from predominant T_H2 cell activity in the newborn period to a more balanced cytokine profile later on.[66,159]

The extended hygiene hypothesis postulates that the induction of T_{REG} cells is an important part of such microbe-driven immunological homeostatis.[33] Naturally occurring T_{REG} cells with suppressive properties are present in large numbers in fetal MLNs[160] and these are probably part of a systemic tolerance to keep autoreactive effector T cells in check to avoid inflammation and tissue damage.[136] These T_{REG} cells are apparently

induced centrally in the thymus and proliferate in peripheral tissues.[161,162] After birth, MLNs and other mucosa-draining lymph nodes may be largely responsible for the cellular decision to induce hyporesponsiveness against an innocuous exogenous antigen versus the decision to induce potentially harmful systemic-type productive immunity. As mentioned previously, the driving force for this homeostatic mechanism seems to be the microbial impact that conditions APCs and T cells for tolerance by balancing polarizing cytokines induced via PRRs (Figures 8 and 9).

MAMPs, therefore, do not only directly modulate the intestinal epithelial barrier function of neonates,[95] but also the activation profiles of innate and adaptive immune cells. Appropriate balancing of the immune system seems to depend on a fine-tuned crosstalk between APCs (innate immunity) and T cells (adaptive immunity) during certain windows of opportunity, particularly soon after birth[33,47] and probably late in fetal life.[163] Concepts such as epigenetic DNA programming (for example, altered methylation) *in utero* and subsequent epigenetic regulation are being considered as critical pathways through which environmental changes could alter the expression of genes that lead to immune homeostasis or dysregulation.[81] T_{REG} cells and the T_H1/T_H2 balance are probably important in this process.[164,165] Reprogramming of the immune system may even be a life-long process (Figure 10). This theory could explain the late emergence of allergy in some individuals and also the difficulty in pinpointing the most important genes that predispose to food allergy, although no genome-wide studies have been published yet.[147]

The high prevalence of immune-mediated diseases in affluent societies indicates that susceptibility genes for immune dysregulation are almost universal, such that they can be induced readily with environmental change. Epigenetics is a new research frontier providing novel understanding of how the environment can have heritable genomic effects and promote disease.[81,147,165] Perinatal exposure to foreign antigens, microbes and dietary nutrients has been shown to have effects on gene expression and influence clinical phenotype. For example, mice born to dams exposed to bacteria during pregnancy were shown to experience less allergy than those born to unexposed dams and maternal TLR signaling was needed for the transmission of this protection.[166] Even the sensitivity of gut epithelial cells to lipopolysaccharide exposure via TLR4 may be subjected to epigenetic regulation.[167]

The heredity of allergy is, however, complex. Concordant peanut allergy is reported to be approximately 65% in identical twins compared with 6–7% in dizyotic twins.[147] Family history still remains the best diagnostic indicator of food allergy, but it can be difficult to obtain a complete and reliable family history.[168] Rough estimates indicate that some 50% of children will become allergic if both parents are atopic, but this figure drops to 25–30% if only one parent is atopic. Nevertheless, for most allergic children there is no positive family history. Hopefully, therefore, the apparently inherent plasticity

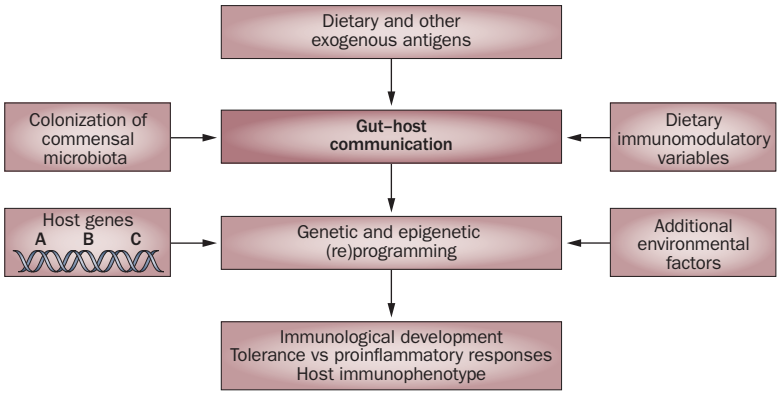

Figure 10 | Influence of the environment on gut–host communication and immunological development. The gut represents a large communication organ that transmits a variety of biological signals from the environment to the host. These signals are essential for immunological development and may determine the balance between tolerance and proinflammatory responses. The interaction of the environment with host genes (A, B and C) provides opportunities not only for programming but also for reprogramming of the immune system. Prevention of allergy may therefore be achieved at various levels by influencing the immunophenotype, partly through epigenetic regulation.

of the immune system may in the near future provide opportunities for reprogramming to provide more effective prevention and treatment of allergies. Even APCs of adults can be conditioned to induce T_{REG} cells by environmental factors, such as lipopolysaccharide[169] and cell wall lipids from parasites.[170] Interestingly, transient infestation of porcine helminths in patients with IBD has been shown to have a beneficial effect on mucosal immune homeostasis,[143] and the same has been shown in experimental models of allergy.[141]

The first postnatal year of life seems to be a key period for programming of the immune system. During this period colonization of the newborn's gut mucosa with commensal vaginal and fecal bacteria derived from the mother's birth canal is normally being established.[171] *In utero*, abortion is associated with a T_H1 response and apparently avoided by a T_H2-skewed cytokine profile[66] that in healthy newborns is then deviated towards a T_H1 profile as part of the immunological maturation.[159] Conversely, in atopic infants, the T_H2 skewing will continue and thus predispose to allergy. The possibility that IgE sensitization may occur *in utero*[169] remains elusive[172] and does not necessarily suggest that the infant will become atopic with subsequent allergy.[173] Instead, the infant may later develop a homeostatic T_H1/T_H2 cytokine profile. For example, most children with cow's milk allergy, particularly those with non-IgE-mediated hypersensitivity, outgrow their disorder before 3 years of age.[3] However, in affluent societies such tolerance adaptation seems to be delayed;[174] 10–25% of infant IgE-mediated milk allergy is retained and hypersensitivity expands to more than one food in 50% of affected children.[3] Importantly, allergy to milk or eggs is associated with an increased risk of sensitization to aeroallergens and the development of asthma.[3] One-third of children with food allergy are reported to have asthma.[175]

Homeostatic impact of commensal gut bacteria

Accumulating evidence supports a central role of indigenous gut bacteria in the extended hygiene hypothesis.[33] The gut microbiota of young children in Sweden was found to contain a relatively large number of *Clostridium* bacteria, whereas high levels of *Lactobacillus* and *Eubacterium* bacteria were detected in an age-matched population from Estonia.[176] This difference might contribute to the lower incidence of allergy in the Baltic countries compared with Scandinavia.[177] A Finnish study likewise reported that infants with allergy had greater numbers of clostridia and fewer bifidobacteria in their stools than nonallergic controls.[178]

Absence of early postnatal colonization of the gut with normal commensal gut bacteria (dominated by lactic-acid-producing bacteria) might also contribute to the increased risk of food allergy that is noted in children delivered by cesarean section, particularly when they have a genetic predisposition to allergy.[96,179,180] As mentioned previously, a clear effect of probiotic and prebiotic perinatal intervention on allergy in general has been difficult to prove, even in children at high risk. Thus, the effect of postnatal synbiotic intervention in newborns until 6 months of age for the prevention of IgE-mediated allergy (including food allergy) is unclear.[181] However, children delivered by cesarean section show a modest but statistically significant reduction when given this regimen.[181] Clinical benefits might, therefore, be achieved by balancing the colonization of the gut microbiota and inducing homeostatic immune regulation.

The feeding (for example, breast milk) and treatment (for example, antibiotics) conditions to which a newborn is subjected and general nutritional state may have an influence on indigenous gut microbiota and on intestinal epithelial integrity. Such variables may modulate the programming of the mucosal immune system.[182,183] Intestinal colonization of lactobacilli and bifidobacteria is promoted by breast milk because it acts as a prebiotic by supplying large amounts of oligosaccharides,[56,57,183] and breast milk may also contain probiotic bacteria.[184]

Cell culture studies have suggested that probiotics could have a direct immunomodulatory effect by enhancing the T_H1 profile via induction of IL-12, IL-18 and IFN-γ secretion.[185,186] Also notably, *E. coli* is a strong inducer of IL-10 secretion derived both from APCs and T_{REG} cells.[187,188] IL-10 has been shown to be an important suppressive cytokine in the murine gut.[189] Importantly, T_{REG} cells bear PRRs for several MAMPs[190] and IL-10 is crucial to maintain expression of the Foxp3 transcription factor,[191] which contributes to the function of these suppressive cells.[138] A defective Foxp3 transcription factor caused by various gene mutations gives rise to immunodysregulation, polyendocrinopathology and enteropathy X-linked syndrome or X-linked autoimmunity-allergic dysregulation.[192] These patients have several organ-specific autoimmune diseases but also increased serum levels of IgE that result in food allergy associated with severe eczema or dermatitis and enterocolitis.

Early allergen exposure and other variables

The data presented so far imply that the gut microbiota has an influence on mucosal homeostasis beyond that of enhancing the SIgA system, namely by promoting a balanced development of T_H1, T_H2, and T_{REG} cells.[193] The induction of various subsets of T_{REG} cells may also be influenced by additional factors, such as hormones.[194] Furthermore, indoor pollution, such as cigarette smoke,[195] and the molecular characteristics of certain allergens[196,197] may skew the cytokine balance in favor of the development of T_H2 responses and IgE-mediated allergy.

Importantly, early allergen exposure may not necessarily have adverse effects. This thinking is a marked change from previous mythological approaches to allergy prevention that focused heavily on early allergen avoidance to prevent sensitization. With the awareness that peripheral T_{REG} cell induction is an antigen-driven process[198] and that mucosal exposure to allergens is not the cause of the allergy epidemic,[199,200] there has been a significant re-evaluation of the many allergen restriction strategies, including delayed induction of complementary feeding in addition to breast milk, and prolonged avoidance of potentially allergenic foods.[201,202]

The American Academy of Pediatrics and the UK Government's Chief Medical Officer's Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, previously suggested that maternal dietary avoidance of strong allergens (such as egg, cow's milk, fish, tree nuts, and particularly peanuts) during pregnancy and lactation might reduce the incidence of allergy in infants at hereditary risk of allergy.[203] However, there is no scientific evidence that conclusively shows that maternal dietary restriction is protective.[202] The guidelines were therefore revised in 2008 to recommend exclusive breastfeeding for only the first 4–6 months of life, and then the introduction of solid food, preferably combined with breast milk.

The potential benefit of allergens as 'tolerogens' in the prenatal and early postnatal period is supported by clinical observations and this new view of immunological homeostasis has been extended to preventive and therapeutic approaches to managing food allergy.[2,3,8,35,37] Food antigens are employed to elicit tolerance in children with food allergy as part of specific oral tolerance induction (SOTI) programmes.[36,204] Similar approaches were introduced 20 years ago but were not evaluated in a placebo-controlled manner. Clinical trials now report a significantly increased threshold of reactivity to various food allergens (for example, egg, cow's milk, and peanut) and a subsequently less restrictive lifestyle in children who receive SOTI.[2,36,204–207] Sublingual immunotherapy has shown similar promise to SOTI in managing food allergy and is evaluated in several clinical trials.[2,36,207] Nevertheless, these approaches remain experimental and carry the risk of inducing adverse reactions. Modifications to make them safer are under way, such as the use of heat-denatured milk proteins and recombinant peanut allergens for SOTI.[208,209]

Children with established food allergies should still continue to avoid culprit foods by adhering to an

elimination diet. However, such diets do carry the risk of providing insufficient supplies of critical nutrients with associated adverse effects on health and well-being. This problem highlights the need for careful supervision of children with food allergy who are on elimination diets. Furthermore, immunological homeostasis is influenced by nutritional factors beyond allergenic proteins (Figure 10).[183] The potential for different dietary immunomodulatory nutrients, such as micronutrients and antioxidant vitamins, to influence immune development and host–microbial interactions is being evaluated.[183,210] Chinese herbal preparations for the prevention of food allergy are also being investigated in clinical trials.[211] Such an approach has gained scientific support after a combination of nine herbs was shown to be highly effective in preventing peanut anaphylaxis in a mouse model;[212] T_H2 responsiveness and allergen-specific IgE was reduced apparently through IFN-γ produced by $CD8^+$ T cells.

Lipid intake, such as fish oil enriched with polyunsaturated omega-3 fatty acids, may protect against food allergy, but not against established allergic disease.[213] Of note, fecal levels of short-chain fatty acids have been shown to be relatively low in children with food allergy and this apparently reflects the slow maturation of the gut microbiota.[214] Lipid-based allergy prevention strategies should, therefore, be performed early, perhaps even *in utero*.[183] Animal experiments have suggested that the ratio of omega-6:omega-3 fatty acids is of particular importance for neonatal oral tolerance induction.[215] This ratio varies in breast milk from women in different parts of the world, a finding that may explain the reported variable effects of breastfeeding on allergy prevention.[216,217] A derivative of omega-3 fatty acids, termed resolvin E1, binds with high affinity to a receptor (ChemR23) on APCs, thereby attenuating NFκB activation.[218] This may explain, at least in part, the apparent anti-inflammatory effect of omega-3 fatty acids.

As mentioned previously, dietary vitamin A may influence mucosal immune maturation because it can be metabolized to retinoic acid by RALDH in intestinal DCs and macrophages.[134] Importantly, retinoic acid enhances the gut-homing properties of T cells and B cells, and promotes B-cell switching to IgA expression in GALT.[32,50,51,134] Moreover, together with certain cytokines, retinoic acid stimulates the induction of T_{REG} cells[136–139] and their homing to the intestinal lamina propria (Figure 8). Vitamin-A-deficient mice exhibit dramatically reduced numbers of T cells, both in the intestinal lamina propria and epithelial compartment.[219]

T_{REG} cells in the control of food allergy

Both thymus-derived, so-called 'natural' $CD4^+CD25^+$ T_{REG} cells and inducible (or adaptive) counterparts are subject to extensive research; a distinction between natural and inducible T_{REG} cells is difficult, although the former subset is clearly essential to avoid autoimmunity.[136] The suppressive function of T_{REG} cells is directed both against the adaptive and the innate arm of the immune system and generally dampens inflammatory reactions.[220] T_{REG} cells are typically anergic upon T-cell receptor stimulation but can proliferate when interacting with appropriate APCs in the presence of IL-2.[221] The conversion of naive T cells to inducible T_{REG} cells may also take place with minute antigen doses in the absence of IL-2.[222] Importantly, the interaction of MAMPs with TLRs on T_{REG} cells (Figures 7 and 9) is involved both in the regulation of the proliferation and the suppressive capacity of these cells.[223] A study of germ-free mice has demonstrated that lipopolysaccharides in the diet provides sufficient TLR stimulation to expand T_{REG} cells in MLNs.[224]

Common phenotypic markers of active $CD4^+CD25^+$ T_{REG} cells, in addition to the high-affinity α-chain (CD25) of IL-2R, are CD45R0, L-selectin (CD62L), CTLA-4 $(CD152)^{high}$, $CD127^{low}$ and glucocorticoid-induced TNF receptor (GITR).[225] Notably, however, several of the same markers can be displayed by activated subsets of effector T cells. Only functional assays can firmly identify $CD4^+CD25^+$ T_{REG} cells. However, research in mice and humans has shown that the fork-head family transcription factor Foxp3 is generally selectively expressed by naturally occurring $CD4^+CD25^+$ T cells and a major subset of peripherally induced $CD4^+CD25^+$ T cells.[136] Although IL-2 may not be required for Foxp3 expression, it has an essential function in peripheral homeostasis mediated by T_{REG} cells.[226]

$CD4^+CD25^+$ T_{REG} cells can employ several mechanisms to suppress immune responses—either via direct cell contact with effector T cells or indirectly by reducing the function of APCs.[136,225] In addition, downregulatory cytokines, such as TGF-β and IL-10, may be involved in the suppressive effects of T_{REG} cells, at least in certain circumstances (Figure 9). Although the details of the regulatory process remain uncertain, evidence suggests that under appropriate conditions one or more of the identified suppressive properties of T_{REG} cells may be acquired by any functionally mature T cell in a normal peripheral immunostimulatory process.[136] Whether various inducible T_{REG} cells represent separate T-cell lineages remains unclear.[225] A study has suggested that certain resting $(Foxp3^{low})$ and active $(Foxp3^{high})$ human T_{REG} cells can be distinguished by their CD45 isotype expression because they are $CD45RA^+$ (naive) and $CD45RA^-$ (that is, $CD45R0^+$; memory), respectively.[227]

Direct evidence for a role of $CD4^+CD25^+$ T_{REG} cells in oral tolerance has been obtained from murine feeding experiments,[79,228–231] and these cells may[232] or may not[233] express Foxp3. Other identified T-cell subsets possibly involved in oral tolerance are T_R1 cells that mainly produce IL-10, T_H3 cells that produce TGF-β, and a subset of cells that express inactive TGF-β as revealed by latency-associated peptide ($T_{REG}LAP^+$) on their surface (Figure 7). In a study of children who outgrew their non-IgE-mediated cow's milk allergy after a milk-free period (>2 months), a population of $CD4^+CD25^+$ T cells with an apparent contact-dependent suppressive activity was found in peripheral blood 1 week after milk exposure was initiated *in vivo*.[234] This subset of T cells was numerically and functionally at a significantly lower level in children

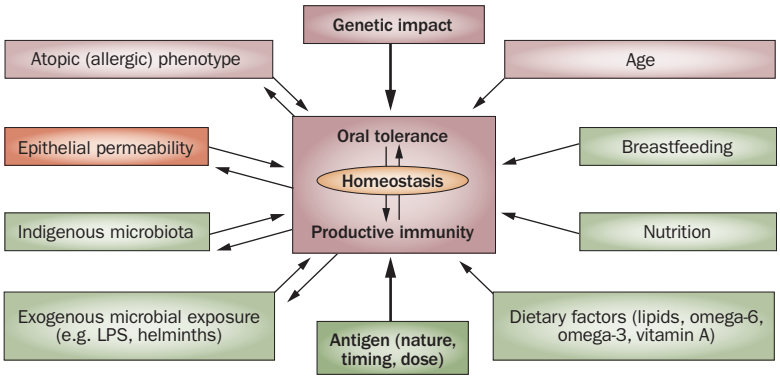

Figure 11 | Biological variables that influence the developing immunophenotype of an infant. Immunological homeostasis depends on the balance between mucosally induced oral tolerance and productive immunity (secretory IgA-mediated and systemic). Several of the components acting on this balance are reciprocally modulated as indicated by bidirectional arrows. The impact of genes and antigen are most important as indicated by the thickness of the arrows. Green boxes represent components that may be subjected to intervention modalities as discussed in the text. The importance of the epithelial barrier function is highlighted in red.

who continued to suffer from allergy. Outgrown food allergy was associated with a lower proliferative activity of circulating effector T cells with specificity for the major milk allergen β-lactoglobulin. This finding apparently reflects the higher frequency of circulating $CD4^+CD25^+$ T_{REG} cells.[234] In these milk-tolerant children, 30% of T_{REG} cells expressed the activation marker CD45R0 whereas the corresponding figure in the persistently food-allergic group was only 5% both before and after milk challenge.

This study provided the first human data to suggest that the induction of oral tolerance to dietary antigens is associated with the development of $CD4^+CD25^+$ T_{REG} cells. The same cellular phenotype has subsequently been identified in the peripheral blood of children who have outgrown IgE-mediated cow's milk allergy.[235,236] The involved $CD4^+CD25$ T_{REG} cells are suggested to employ Foxp3, CTLA-4, or IL-10 as suppressive molecules.[236,237]

Current and future perspectives

Many variables influence the induction of oral tolerance and productive SIgA-dependent mucosal immunity. Some of these variables are reciprocally modulated to achieve mucosal immune homeostasis (Figure 11). Increased epithelial permeability for exogenous antigens is clearly an important primary or secondary event in the pathogenesis of many diseases, including food allergy (Figure 5). Postnatal epithelial barrier function is determined by a newborn's age (for example, preterm versus full term), genetics, mucus composition, interactions between mast cells, nerves and neuropeptides, concurrent infection, and the mucosa-shielding effect of SIgA provided by breast milk or produced in the infant's gut. Furthermore, the integrity of the intestinal epithelium depends on homeostatic mechanisms, such as the induction of T_{REG} cells.

The incidence of food allergy is suggested to be increased in individuals with delayed or impaired development of the IgA system.[56] An underlying deficiency of antigen-specific SIgA has been proposed in a mouse model of food allergy.[238] This finding implies the involvement of secretory antibodies in oral tolerance induction. Another experimental model investigated the relation between oral tolerance and hypersensitivity in the presence of a defective intestinal surface barrier due to SIgA/SIgM deficiency.[79] This deficiency leads to systemic hyper-reactivity of the host, but at the same time also enhances the induction of oral tolerance in a delicate balance (Figure 4). Boirivant *et al.*[233] have reported similar findings. A mild or transient breaching of zonula occludens in the intestinal epithelium of mice leads to a dominant anti-inflammatory T_{REG} cell response. The relatively leaky intestinal epithelium of newborns could thus promote the induction of oral tolerance against luminal antigens and the homeostatic balance is enhanced by cognate SIgA antibodies. It is, therefore, not surprising that several epidemiological reports suggest that breastfeeding protects against allergy. The remarkable output of SIgA during breastfeeding represents optimally targeted passive immunization of the breastfed infant's gut[56,57] and might serve as a positive homeostatic feedback loop (Figure 6).

The secretory immune system is critical for epithelial barrier function because SIgA not only forms the first line of adaptive defense, but also maintains mutualism with the indigenous gut microbiota. Notably, epithelial barrier function depends on exposure to MAMPs and the induction of oral tolerance via mechanisms such as tolerogenic APCs and T_{REG} cells (Figure 9). It has, therefore, been proposed that the 'the hygiene hypothesis' should instead be called 'the microbial deprivation hypothesis'.[34] In mouse experiments it has indeed been shown that a single immunomodulatory molecule from a commensal gut bacterium can induce crucial modulation and homeostasis of the host's immune system.[239] Accordingly, the treatment of gastric ulcer by use of antibiotic eradication of *Helicobacter pylori* has been suggested as a potential risk factor for allergic disease.[240]

It would, however, be premature to conclude that improved hygienic measures and the use of antibiotics fully explain the increased incidence of food allergies in affluent societies. There are other potential allergy-promoting risks in a modern lifestyle. For example, gastric-acid-suppressive drugs are sold abundantly and used largely in an inappropriate manner as antiulcer medication.[241] Such medication reportedly increases the risk of food allergy because a fraction of dietary allergens (for example, bovine β-lactoglobulin and peanut proteins) are quite resistant to digestion,[2] which is inhibited by the drugs.[241] Notably, gastric proteolysis is optimal at pH 1.0–3.0. Children up to 2 years of age have an increased gastric pH and are, therefore, normally exposed to relatively more intact food proteins, which may contribute to their predisposition to food allergy.

In Australia it has been observed that the prevalence of food allergy (most commonly against peanuts, egg, cow's milk, and cashew nuts) among children aged 0–5 years has increased by a factor of 12 between 1995 and 2006.[242]

This cannot be explained by the general increased gastric pH found in children. The increase of food allergy in the US is reported to be at least twofold over this same period.[8] One may, therefore, wonder if there is something particular in the diet of these countries that unduly and continuously reduces the capacity of the intestinal epithelial barrier to protect against major allergens. There has been progressive development of genetically modified (GM) maize and soy over the past decade and GM maize now accounts for 80% of maize production in the US. Although GM food is generally considered safe and to have no increased allergenic potential,[243] animal experiments suggest that the transgenic toxins and enzymes in these products may variably affect mucosal and peripheral immune responsiveness depending on the age of the animal.[244–246] The induction of IgG antibodies to the actual food antigen and even crosspriming against a bystander antigen may be of biological significance. Experimental studies both *in vitro*[247] and *in vivo*[248] have demonstrated that IgG antibodies that are not balanced by a mucosal IgA response can enhance the epithelial penetration of bystander proteins. Further studies are needed to determine whether this phenomenon could have relevance to the allergy epidemic.[113]

Clinical evidence suggests that skin permeability is also relevant to sensitization against food antigens.[8] This hypothesis explains the association between severe eczema in infancy and the development of food allergies. A dose–response relationship has been observed between household (environmental, nonoral) peanut exposure and the incidence of peanut allergy.[200] Atopic eczema remains an enigmatic inflammatory disorder that involves complex interactions between the environment and susceptibility genes that encode skin barrier molecules.[249] Keratinocytes secrete a unique profile of chemokines and cytokines after activation and are a particularly rich source of thymic stromal lymphopoietin.[249] Interestingly, thymic stromal lymphopoietin induces the expression of OX40L on DCs and the engagement of this and other signaling pathways drives the differentiation of inflammatory T_H2 cells.[250] Inflamed skin that is exposed to dietary allergens may, therefore, be a probable origin of food allergy and peanut-containing skin ointments should clearly be avoided.

Another potential predisposing variable is obesity. It is well documented in the US National Health and Nutrition Examination Survey that obesity in children is significantly associated with markers of generalized inflammation and the severity of asthma.[251] Clusters of lymphoid cells are seen both in human and mouse fatty tissue; in mice these clusters were recently shown to contain a novel T_H2-type innate lymphocyte.[252] These cells release large amounts of IL-5 and IL-13 and may therefore represent a link between obesity and IgE-mediated allergy.

Conclusions

Food allergy is considered to be a major health concern and seems to be epidemic in some affluent parts of the world. Although much evidence suggests that early microbial stimuli are critical for immune development, further studies are needed to determine the role of altered MAMP exposure in the pathogenesis of this disorder. The events that initiate food allergy are probably multifactorial and immunological problems associated with certain relatively recent dietary antigens are not surprising from an evolutionary perspective.

Data from the large German Infant Nutritional Intervention Study and other reports suggest that there is a modest long-term preventive effect of hydrolyzed infant formulas on allergic manifestations in children at high risk of food allergies.[253] In individuals with established food allergy, however, elimination diets remain the only well-proven form of therapy. Novel management strategies, such as improved SOTI,[254] will probably emerge in the near future with attempts to induce homeostatic immune regulation.[255] These strategies may be either generalized approaches to suppress immune hypersensitivity to all potential dietary allergens or allergen-specific approaches. Along the first line, molecular refinement of probiotic and prebiotic intervention is an exciting avenue for further research.[210] Novel bacterial or parasitic molecules may be developed to enhance the precarious balance between tolerance and immune dysregulation. Along the second line, the intention is to induce tolerance to selected allergen(s) by low-grade and relatively early exposure through the gut. This strategy is in sharp contrast to the long-lived myth about total allergen avoidance as a preventive measure in families at high risk of allergy. Heat-denatured and genetically engineered allergen peptides may aid this promising approach.

Review criteria

The PubMed (MEDLINE) database was searched using the terms "food allergy", "atopic eczema", "atopic dermatitis", "anaphylaxis", "oral tolerance", and "regulatory T cells" for articles published between 2008 and 2010. Relevant papers were selected first on the basis of abstracts, then on the basis of full-text articles in English. Relevant papers published before 2008 were either familiar to the author or they were selected by searching the reference lists of identified papers.

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